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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Dorit PLAT et al.	Confirmation No. 3410
Serial No.:	10/572,782	Examiner: Abigail L. FISHER
Filed:	November 8, 2006	Art Unit: 1616
For:	STABILIZED FORMULATIONS OF PHOSPHATIDYLSERINE	

DECLARATION OF NETA SCHEINMAN UNDER 37 C.F.R. § 1.132

In the Matter of

US Patent Application Ser. No. 10/572,782

Stabilized Formulations of Phosphatidylserine (hereafter "**the patent application**")

I, Neta Scheinman, hereby declare and state as follows:

I am a chemical engineer since the year 2000. I have been employed by Enzymotec Ltd. since the year 2001, and am currently employed as the Quality Manager of Enzymotec Plant. My CV is attached hereto (**Annex A**).

I am a co-inventor of the invention subject of the above-identified application.

I have read and understand the patent application. I have also read and understand the amended claims, attached hereto, to be filed with this declaration.

The invention recited in amended claims relates to a phosphatidylserine (PS) composition of matter comprising an oil base and from about 1 to about 99% (w/w) PS, wherein the PS is predominantly in the form of its salt with a divalent ion and is dispersed in the oil base. The composition exhibits a stability of less than about 1 to about 5% decomposition of the PS after a

storage period of at least 6 months. The divalent ion is particularly calcium or magnesium. This aspect of the invention is referred to hereinafter as "the dispersion of the invention". The invention of the patent application, as claimed, also relates to a combination of a capsule with the dispersion of the invention contained in the capsule. This aspect of the invention is referred to as hereinafter as "the capsules of the invention".

In order to compare the storage stability of the dispersion of the invention with commercially available PS (contained in PS capsules), the following experiments were conducted at my request and under my direct supervision and control. I participated in the design of and approved all experiments. I continuously monitored the experiments in order to assure that they would be carried out according to their design.

Shelf-life studies included both standard (ambient) conditions (Room Temperature) and "accelerated" or stress conditions (35°C, 60% relative humidity).

1. Long-term stability of PS calcium salt dispersion contained in soft gel capsules

A. Preparation of the PS calcium salt was carried out in the manner set forth in the patent application, and proceeded as follows:

1. Purified vegetable source lecithin (Phospholipid, Germany) was incubated in a stainless still reactor with an excess of L-serine (Kyowa, Japan) in the presence of an acetate buffer solution containing $CaCl_2$ (pH=5.6), at 40°C. Transphosphatidylation was carried out by the addition of phospholipase D from microbial source for 24 hours, at 40°C.
2. After completion of the reaction, the mixture was separated in a 1.2 m diameter basket centrifuge.
3. The separated PS calcium salt product was washed with deionized water.
4. The water-washed PS calcium salt product was washed with ethanol at an elevated temperature of 60-70°C.

5. After cooling to 25°C the obtained PS salt product was separated using a 1.2 m diameter basket centrifuge.
6. The separated PS salt product was dried in a vacuum tray dryer at 50°C and vacuum of 5 mbar to obtain a dried powder.
7. The dried powder was dispersed in medium-chain-triglycerides (MCT) oil (by SternChemi, Germany) to form a flowable liquid product.
8. The flowable liquid product of step 7, consisting of the PS calcium salt powder obtained in step 6, dispersed in MCT as in step 7, was encapsulated in soft gel capsules by a third party company, specializing in encapsulation (Factors Group of Nutritional Companies Inc., Canada).

B. Experiment conditions of stability analysis:

9. A first portion of the PS-containing capsules obtained as above were stored at ambient conditions in a closed package and placed in a dark place at room temperature.

10. A second portion of the PS-containing capsules obtained as above were stored at accelerated conditions in a closed package and placed in an incubator at 35°C and 60% relative humidity.

11. The content of the capsules (both portions), i.e. the PS calcium salt obtained in step 6 above dispersed in medium-chain-triglycerides (MCT) oil was analyzed for PS content, using ^{31}P-NMR, by the Spectral Service Laboratorium für Auftraganalytik GmbH. Annexed hereto are the Certificate of GMP-Compliance (attached as **Annex B**) and Certificate of GLP-Compliance (attached as **Annex C**). This laboratory is the official laboratory of the International Lecithin and Phospholipids Society (ILPS) and is considered by every phospholipids manufacturer as the most competent and reliable choice. The origin or identity of each capsule or sample was not known to the laboratory.

13. Original report from the laboratory is annexed (attached as **Annex D**).

14. As can be seen from Figure 1 and Table 1, the PS content of the composition in the capsules containing the PS dispersion prepared in accordance with the invention recited in the amended claims, following long-term storage at ambient conditions was almost identical to

baseline values even after 24 months of storage. The level of PS in the composition in capsules kept at accelerated conditions was very similar to that of the level of PS in the composition in the capsules kept at ambient conditions. These results show that the PS dispersion prepared in accordance with the invention recited in the amended claims has very long shelf-life, and no decrease in PS levels was observed even after 2 years of storage.

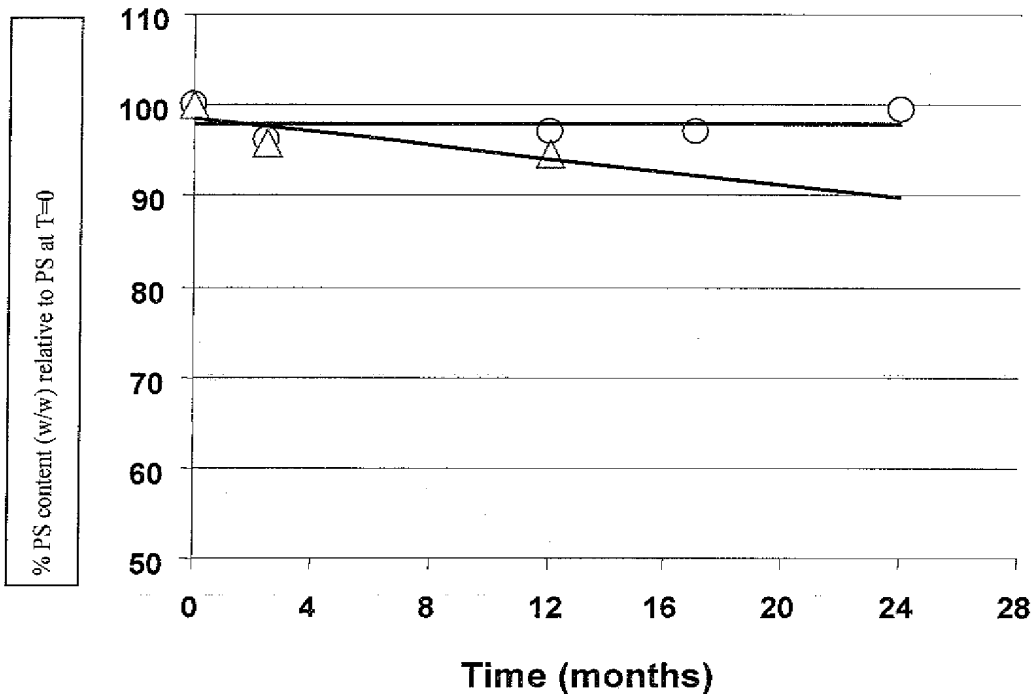

Figure 1: ^{31}P-NMR analysis results for PS content of soft gel capsules of the invention at ambient (○) and accelerated (△) storage conditions over 24 and 12 months, respectively.

Raw data is provided in Table 1.

Table 1: ^{31}P-NMR analysis results for PS content in the composition encapsulated in the soft gel capsules as recited in the amended claims of the invention at ambient and "accelerated" storage conditions over a 24 month period. Results are expressed as % PS content (w/w) relative to PS content (w/w) at T=0

Time (Months)	**Ambient (%PS)**	**Accelerated conditions (%PS mg)**
T=0	100	100
2.5	96.2	95.9
12	96.9	94.5
17	97	--------
24	99.3	--------

2. Comparative results

A commercially available PS product of Degussa (herein "Supplier A"), which is sold under the guarantee of extended shelf life, was also tested (PS capsules by Degussa, sold under LECI-PS® 20V).

As seen in Figure 2, commercially available PS soft gel capsules of supplier A were tested under accelerated conditions and compared to the PS soft gel capsules of the invention as recited in the amended claims.

As shown by this study, PS capsules by the supplier A, advertising its product to be storage-stable, failed to achieve the stability of the PS capsules of the invention as recited in the amended claims ("Stabilized PS dispersion").

Original report from the laboratory is annexed (**Annex E**).

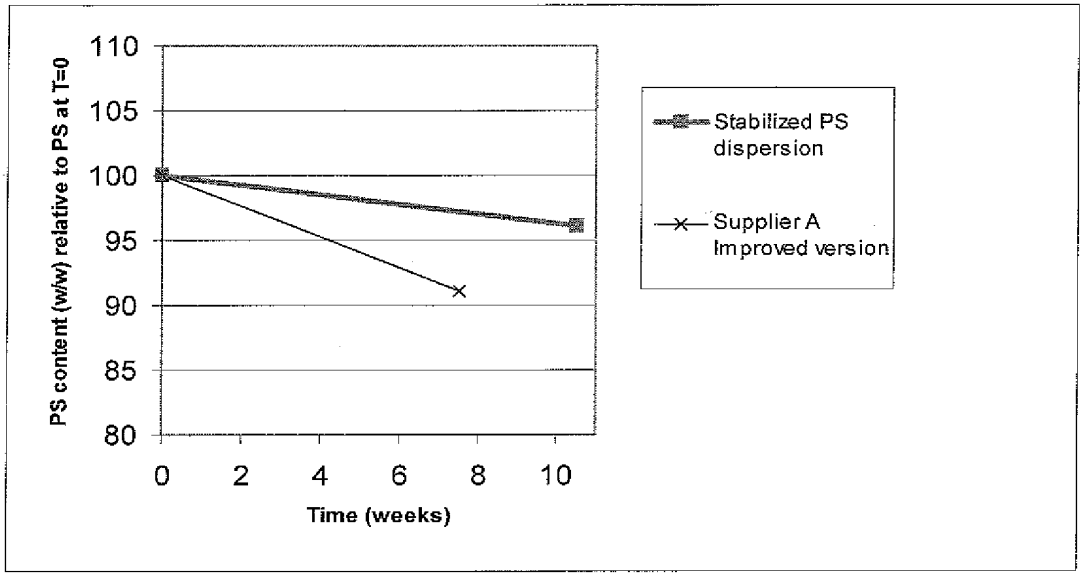

Figure 2: A comparative ^{31}P-NMR analysis of PS dispersion capsules of the invention as recited in the amended claims in comparison with commercial PS capsules advertised to be storage-stable (supplier A's improved version). Capsules were kept under accelerated conditions.

Spectral tests were conducted by the Spectral Service Laboratorium für Auftraganalytik GmbH. Annexed hereto are the Certificate of GMP-Compliance (**Annex B**) and Certificate of GLP-Compliance (**Annex C**).

Cation analysis

The PS in the capsules of the invention as recited in the amended claims and in the commercially available PS capsules of supplier A (stability of both of which is shown in Figure 2), were tested for their metal content, by ICP analysis of sodium, potassium, calcium and magnesium. Analyses were conducted by AminoLab LTD, Israel, which is accredited under ISO/IEC 17025 with standard operating procedures.

Report for analysis of the stabilized PS dispersion of the invention as recited in the amended claims is annexed as **Annex F.** The report for the commercial PS capsules of supplier A (improved, as in Figure 2) is annexed as **Annex G.**

As can be seen from Table 2, the cations found in the commercial PS capsules of supplier A were predominantly sodium and potassium, indicating that the PS exists as a monovalent salt. The cations found in the PS capsules of the invention, as recited in the amended claims, were predominantly calcium, indicating that the PS exists as a divalent salt.

Test	**Supplier A improved version**	**Stabilized PS dispersion**
Calcium (mg/kg)	305	5026
Potassium (mg/kg)	2592	13
Magnesium (mg/kg)	363	13
Sodium (mg/kg)	5202	140

Table 2: A metal scan by ICP of stabilized PS dispersion capsules (according to the invention) in comparison with commercial PS capsules (claiming to be storage-stable, supplier A's improved version).

The undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing from the application referenced herein.

Date: 15/12/2009 By: ENZYMOTEC LTD.
Neta Scheinman